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mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

Severe fungal sclerokeratitis caused by *Metarhizium anisopliae*: a case report and literature review

Hiroshi Eguchi,[1] Toshi Toibana,[2] Fumika Hotta,[1] Tatsuro Miyamoto,[1] Yoshinori Mitamura[1] and Takashi Yaguchi[3]

[1]*Department of Ophthalmology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan,* [2]*Department of Ophthalmology, Takamatsu Municipal Hospital, Takamatsu, Japan and* [3]*Department of Mycosis Research, Management Unit of Division of Bio-resources, Medical Mycology Research Center Chiba University, Chiba, Japan*

Summary

To date, there has been only one published report on the infectious sclerokeratitis caused by *Metarhizium anisopliae*, which is an entomopathogenic fungus. Regarding corneal infection, three reports have been published to date. Although the prognoses of the corneal infections are favourable, prognosis when scleral infection is involved is very poor. A 76-year-old patient presented with foreign body sensation in the left eye. Microscopic examination with Fungi Flora Y staining of the corneal scraping revealed fungal infection. The conjunctiva was melted by the infection over a wide area. Although intensive medications were administered, an emergency surgery was necessary because scleral thinning, corneal perforation and lens prolapse occurred. The fungal isolate was identified as *M. anisopliae* by sequencing the internal transcribed spacer region. Herein, we report the second known case worldwide of *M. anisopliae* sclerokeratitis, and we review the literature related to the ocular infections.

Key words: *Metarhizium anisopliae*, sclerokeratitis, ocular infections.

Introduction

Metarhizium anisopliae is a ubiquitous and entomopathogenic filamentous fungus, which inhabits soil worldwide. However, there are few reports of *M. anisopliae* infections on human tissue, presumably because the optimal temperature for fungal growth is less than 35 °C. Based on this assumption, there is an increased susceptibility to infection on the ocular surface, because its temperature is slightly less than 35 °C.[1] With regard to ocular infections, three reports of keratitis[2–4] and only one report of sclerokeratitis[5] have been published to date.

In terms of visual prognosis, all published cases, including the current case, of *M. anisopliae* ocular infections can be divided into two categories: (A) keratitis in young healthy contact lens wearers, and (B) sclerokeratitis in relatively older patients, following administration of topical steroids. Although visual prognosis of category (A) infection is favourable, that of (B) is very poor. Reviewing the four published articles and the clinical course of the current case, we discovered specific traits of the two categories and some common characteristics in all cases. Herein, we report the second known case of *M. anisopliae* sclerokeratitis, and we review all published ocular infections caused by *M. anisopliae*.

Correspondence: H. Eguchi, MD, PhD, Department of Ophthalmology, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15, Kuramoto-cho, Tokushima-shi 770-8503, Japan.
Tel.: +81 88 633 7163. Fax: +81 88 631 4848.
E-mail: hiroegu0113@gmail.com

Submitted for publication 31 July 2014
Revised 5 November 2014
Accepted for publication 7 November 2014

Case presentation

A 76-year-old male, who lived in southwest Japan (a rural area of the Tokushima prefecture) and had no record of overseas travel, presented to a medical practitioner in December 2012 with foreign body sensation in the left eye. Systemic steroid had been administered for several years, because of a past history of chronic

doi:10.1111/myc.12279
Mycoses, 2015, **58**, 88–92

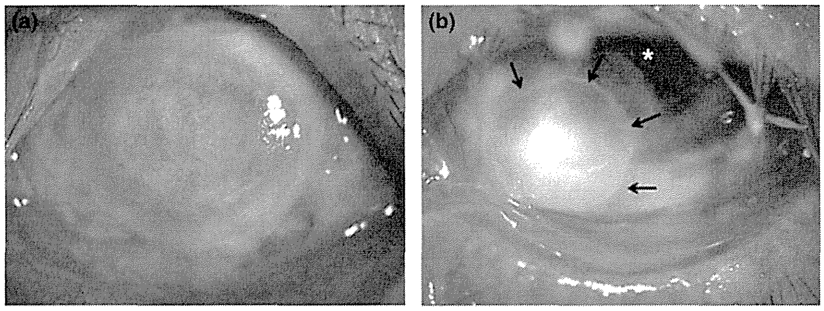

Figure 1 Images of the anterior segments. (a) At patient's first hospital visit: the entire cornea appears white, because the infection spread extensively. The bulbar conjunctiva was melted and was not hyperaemic. (b) On 4 days after the initiation of antifungal medications: a large corneal perforation (white asterisk) and the spontaneous lens prolapse (black arrows) are visible.

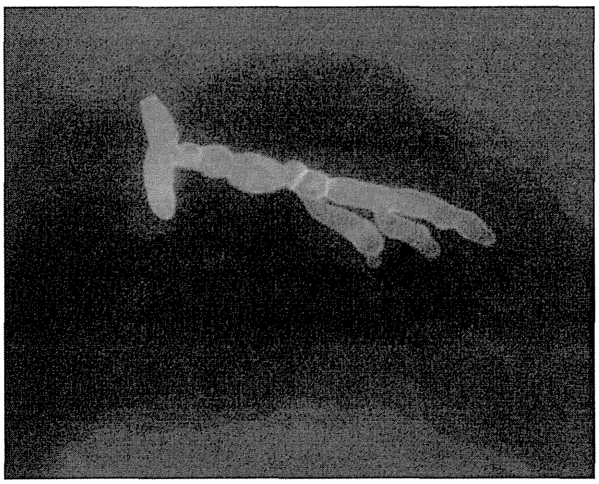

Figure 2 Image of the corneal scraping smear. Fungus was found in the corneal scraping (Fungi Flora Y staining, ×400).

rheumatoid arthritis. The primary care physician diagnosed the case as a corneal ulcer caused by immune reaction related to rheumatoid arthritis. After topical steroid administration, corneal scraping, and soft contact lens wear, the patient was referred to Tokushima University Hospital, because the clinical symptoms worsened.

At the first visit to our hospital, conjunctival hyperaemia was relatively mild than usual fungal keratitis cases, because of the melting. The entire cornea had an ulcer and a stromal opacity. Although the focal corneal thinning was presumed, the thickness of the cornea and the detail of the anterior chamber were unclear. (Fig. 1a). On the nasal side of the ocular surface, necrosis of both the sclera and the limbus were evident. The visual acuity was light perception. The intraocular pressure of the left eye was unmeasurable. We obtained informed consent from the patient before the treatment. Microscopic examination with Fungi Flora Y staining of the corneal scraping revealed fungi (Fig. 2). Although the initial medications were administered, e.g. 1.0% voriconazole eye drops every 1 h, 1.0% natamycin ointment five times daily, oral itraconazole 200 and 100 mg day^{-1}intravenous micafungin sodium, a large corneal perforation occurred 4 days after the initial treatment. Because the lens prolapsed spontaneously through the perforation and scleral melting progressed (Fig. 1b), an emergency tectonic keratoplasty was performed that day.

We obtained a pathogenic strain from the corneal scraping and observed macroscopically and microscopically. The colony on a potato dextrose agar (PDA; Difco, Becton, Dickinson and Company, Sparks, NV, USA) attained a diameter of 30 mm at 25 °C, for 10 days (Fig. 3a). Conidiophores formed a sporulating layer and conidia were produced in long chain from phialides, cylindrical and olive-green in mass (Fig. 3b). As a result of a BLAST search using the sequence of the ribosomal RNA gene internal transcribed spacer domain base, the sequence showed 100% similarity to those from some strains of *M. anisopliae*. Therefore, the isolate was identified as *M. anisopliae* on morphology and phylogeny. The pathogenic strain in the current case was deposited to the Medical Mycology Center, Chiba University in Japan as IFM 61840, and the ITS sequence was submitted to the DNA Data Bank of Japan as LC008306. Minimum inhibitory concentrations (MICs) of several antifungal drugs and the minimal effective concentration of micafungin to the strain were determined by using of the broth dilution method according to M38-A2 of the Clinical and Laboratory Standards Institute (Table 1). After multiple keratoplasties, including several penetrating keratoplasties, scleral transplantation and limbal transplantation plus amniotic membrane transplantation, the transplanted tissue were rejected, and the patient has lost vision 18 months after the first visit.

Discussion

Review of published cases and the current case are shown in Table 2. With regard to the pathogenesis, contact lens wear is most likely the cause of the

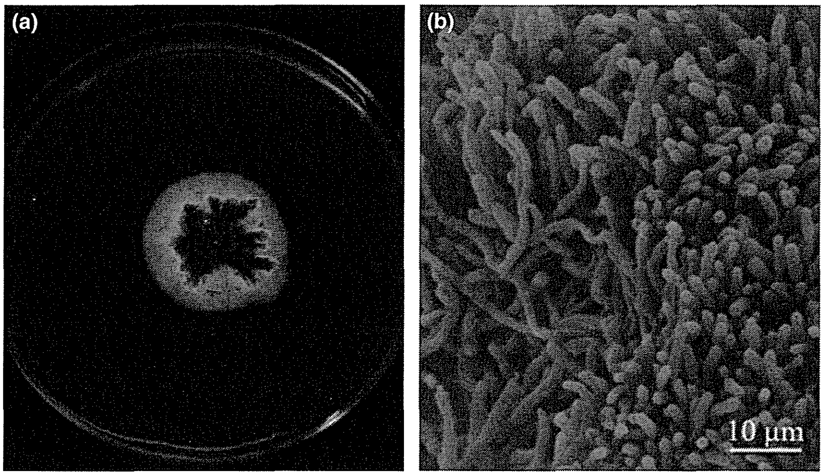

Figure 3 Images of *Metarhizium anisopliae* IFM 61840 inoculated on agar plate. (a) Colony on potato dextrose agar (PDA): a characteristic white olive-green colony on a PDA attained a diameter of 30 mm at 25 °C, for 10 days. (b) Conidiogeneses under scanning electron microscope (bar = 10 μm): condiophores formed a sporulating layer and conidia were produced in long chain from phialides, cylindrical and olive-green in mass.

Table 1 Minimum inhibitory concentrations (MIC) or minimal effective concentration (MEC).

Drugs	MIC or MEC (μg ml^{-1})
Micafungin	<0.015
Amphotericin B	16
Flucytosine	>64
Fluconazole	8
Itraconazole	1
Voriconazole	0.5
Miconazole	1

infection in category (A) patients, although no evidence of *M. anisopliae* isolation from lenses or lens accessories was reported in those cases.[3,4] No history of the usage of commercial products for biological control, including *M. anisopliae*, was reported for either of the two category (B) patients. It is reasonable to conclude these two patients were exposed to the fungus in soil during their work.

A large corneal disorder may reflect the possibility of keratitis progression to the adjacent sclera, and scleral infection causes severe complications requiring surgical intervention or additional treatment.[5] The corneal disorder size can be a predictive factor of visual prognosis for keratitis caused by *M. anisopliae*. Generally, steroids worsen inflammation in fungal keratitis,[6] which would explain the large corneal disorders observed in previous cases. Thus, steroid medications in primary care contributed to large corneal disorders that progressed to adjacent sclera. This progression likely caused eventual poor visual prognoses for the two cases in category (B). In the current case, the past history of chronic arthritis likely complicated the primary care physician's optimal diagnosis. Moreover, several years of previous systemic steroid treatment and soft contact lens wear after the corneal scraping probably impaired the prognosis.

The temperatures of the environments are regulated by the climate and affect the growth of fungi. Patients in the previous reports and in the current case resided in areas of extratropical or polar climates, e.g. Santafé de Bogotá, Colombia[2] has a subtropical highland climate (Köppen-Geiger climate classification; Köppen *Cfb*); Ohio, USA[3,4] has a humid continental climate (Köppen *Dfa*); southeast Australia[5] has a temperate humid climate (Köppen *Cfa*) or marine west coast climate (Köppen *Cfb*); and Tokushima, Japan, has a temperate humid climate (Köppen *Cfa*). Fungal keratitis is thought to be common in tropical and subtropical regions in the world.[7,8] However, all cases in both (A) and (B) categories were reported from the institutions located in extratropical climates. Because much of the human population lives in temperate climate regions, it is critical to understand fungal species that prefer <35 °C for growth and inhabit temperate climate regions, such as *M. anisopliae*, as a pathogen causing ocular infections.

Several reasons account for the poor response in the current case: misdiagnosis as a corneal ulcer caused by immune reaction, use of topical steroids and contact lens wear after the corneal scraping. It will be necessary to accumulate similar case reports of eye infections and establish MICs of several antifungal drugs for those ophthalmic clinical isolates to determine of the optimal treatment for *M. anisopliae* keratitis, and sclerokeratitis. At present, topical natamycin should be administered, because all patients in category (A) received topical natamycin, whereas the MICs for the strains have not been determined. To isolate clinical strains, ocular tissue should be inoculated onto

Table 2 Global literature review of four *Metarhizium anisopliae* ocular infections cases reported so far.

Reference	Category	Country (habitat)	Age	Sex	Eye	Occupation	Onset[1]	Medications at primary physician[2]	Lesions[3] (mm)	Culture condition
De García *et al.* [2]	A	Colombia (Santafé de Bogotá)	18	M	R	NS	NS	NS	4.0 × 3.0 Round shape	Sabouraud, 25 °C A few days
Jani *et al.* [3]	A	USA (Cleveland, OH)	36	F	R	Librarian	9 November	T-Ofloxacin T-Bacitracin/ Poly-myxin B	5.0 × 2.5 Inferior	Potato flakes, 25 °C Sabouraud, 25 °C 3 weeks
Amiel *et al.* [5]	B	Australia (southeastern area)	52	F	R	Cattle rancher	26 July	T-Fluorometholone T-Gentamicin	7.0 × 4.5 Oval Nasal	NS
Motley *et al.* [4]	A	USA (Cincinnati, OH)	12	F	R	NS	NS	T-Amphotericin B	2.0 Round shape Inferior nasal	Sabouraud 5 days
Present report	B	Japan (Tokushima)	76	M	L	Poultry farmer	2 November	T-Betamethasone T-Levofloxacin S-Prednisolone	12.0 × 11.0 Entire cornea	Sabouraud, 25 °C Potato dextrose, 25 °C Sheep blood, 25 °C 10 days

R, right; L, left; NS, not stated; T-, topical administration; S-, systemic administration; M, male; F, female.

[1]Denotes date of symptom occurrence.

[2]Medications administered just before visit to consultants.

[3]Denotes the sizes, shapes and regions of the corneal disorders as far as we know.

a Sabouraud agar plate and cultured at 25 °C. The characteristic white olive-green appearance of a giant colony indicates *M. anisopliae* species.

None of the previous reports described antifungal drug susceptibilities of *M. anisopliae* strains isolated from the ocular infections. In the report by Jani *et al.* [3] the drug susceptibilities based on the MICs for a clinical isolate from another organ were stated; the isolate was susceptible to amphotericin B, flucytosine, fluconazole and itraconazole. To our knowledge, this is the first report of drug susceptibilities with MICs for an ophthalmic clinical isolate of *M. anisopliae.* Although our results show that MICs of antifungal drugs that we used were relatively low, the prognosis of the patient was very poor. The MICs for antifungal agents to clinical isolates does not always reflect the clinical course in fungal keratitis. The surgical approach, such as conjunctival excision, may contribute the high penetration of antifungal agents into the sclera,[9] and the results could differ from this case if we selected it. However, the accumulation of the detailed fungal keratitis cases with both rigorous species identification and MIC or minimal effective concentration for several antifungal agents is crucial for the future cases.

To date, infections occurred equally in male and female. Presumably, it is coincidental that right eyes were infected in the previous reports, because there is no objective evidence suggesting that the right eye is compromised. In two cases[3,5] and the current case, infections were occurred in winter.

In conclusion, *M. anisopliae* can cause infections on the ocular surface of patients living in temperate climate zones. Keratitis is likely to occur in young contact lens wearers. The prognosis of keratitis is favourable when topical natamycin is administered in the early phase of the disease. Sclerokeratitis is likely to occur in elderly patients who engage in agricultural work in rural areas. Unfortunately, the prognosis of sclerokeratitis is poor even if patients receive adequate administrations of antifungal drugs.

Conflict of interest

The authors have no conflicts of interest to disclose.

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